REMARKS

In the Final Action dated December 18, 2002, claims 1-18, 20-26, 29-36 and 38-46 are pending. Claims 1-18, 29-36 and 45-46 are withdrawn from consideration as allegedly drawn to non-elected subject matter. Claims 20-26 and 38-44 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Thomson et al. Science 282:1145-1147 (1998). Claims 19-26 and 37-44 are rejected under 35 U.S.C. §102(e) as allegedly anticipated by Thomson, U.S. Patent No. 6,200,806.

Pursuant to 37 C.F.R. §1.116, Applicants filed a response to the Final Action on April 17, 2003. In response, the Examiner issued an Advisory Action on May 01, 2003, stating that the §1.116 Response has been considered but does not place the application in condition for allowance. Applicants filed a Notice of Appeal on June 18, 2003.

Applicants, through the undersigned, wish to thank Examiner Woitach for the courtesy and assistance extended on behalf of the Applicants during a telephone interview conducted on May 21, 2003. Consistent with the discussion during the interview, Applicants are providing herewith a Declaration of Dr. Martin F. Pera under §1.132. By way of the instant amendment, Applicants have also added new claims 47-52 to more clearly delineate the unique features of the present invention. Applicants have also canceled, without prejudice, claims 1-18, 29-36 and 45-46, allegedly drawn to non-elected subject matter. Applicants reserve the right to file one or more divisional applications to pursue the non-elected subject matter. It is believed that this Response addresses each of the Examiner's rejections in the Final Action and the issues the Examiner raised in the Advisory Action. Accordingly, the present application is condition for allowance. Favorable consideration of all pending claims is respectfully requested.

Claims 20-26 and 38-44 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Thomson et al. (Science 1998). Claims 19-26 and 37-44 are rejected under 35 U.S.C. §102(e) as allegedly anticipated by Thomson (U.S. Patent No. 6,200,806).

Applicants observe that the Examiner's reasons are the same for both the rejection based on Thomson (1998) and the rejection based on U.S. Patent 6,200,806 to Thomson et al. Therefore, Applicants address both rejections together and refer to the two Thomson references as "Thomson et al."

Applicants previously submitted that the methods taught by Thomson et al. do not point to the importance of the feeder cells for the differentiation of ES cells. In the Final Action, the Examiner states, however, that Thomson et al. do teach the importance of feeder cells for the state of differentiation of human and primate cells in that Thomson et al. purportedly teach that, unlike mouse ES cells, feeder cells are required for maintaining the undifferentiated state of primate and human ES cells. Applicants also submitted that Thomson et al. only teach the spontaneous differentiation of ES cell, not the specific induction of differentiation. The Examiner contends, however, that Thomson et al. specifically teach the parameters that affect the differentiation of the cell lines, including the feeder layer, the cell density, and various growth factors such as LIF. The Examiner also contends that it is known that if the ES cells are allowed to grow at high density, differentiation of the ES cells will occur. Therefore, it is the Examiner's opinion that the present claims do not recite method steps which are different from those disclosed by Thomson et al. The Examiner acknowledges that the differentiation disclosed by Thomson et al., could be viewed as spontaneous to the extent that the specific factors, which induce the cells to differentiate, are not specifically known. However, it is the Examiner's opinion that neither the instant claims nor the present specification teach those unknown factors.

In response to the Examiner's contentions as set forth in the Final Action, Applicants respectfully submit that the present claims are directed to an *in vitro* method of inducing somatic differentiation of undifferentiated, pluripotent human embryonic stem cells by growing the stem cells under culture conditions that induce somatic differentiation.

As described in the specification on page 20, there are some fibroblast feeder layers which can induce differentiation in either the extra embryonic differentiation or somatic differentiation streams. On page 20, lines 23 to 25, the specification further teaches that somatic differentiation *in vitro* of the ES cell lines is a function of (i) the period of cultivation following subculture, (ii) the density of the culture and (iii) the fibroblast feeder cell layer. Specifically, the specification has provided the conditions that induce somatic differentiation, contrary to the Examiner's allegation. Such conditions include a combination of (i) feeders that could support proliferation and renewal of stem cells with (ii) prolonged cultivation and/or high density that did not kill the cells, but restricted their self renewal and directed them to differentiate towards somatic tissues. These conditions are presently recited in the claims.

Applicants respectfully submit that the Thompson et al. citations do not provide any insight that the type of fibroblast feeder layer can affect the outcome of the differentiation. Thomson et al. teach that feeder layers help maintain undifferentiated cells, but do not appreciate that various feeder layers can influence somatic or extra embryonic differentiation. In contrast, as stated on page 20, line 19 and page 21, line 22 of the specification, the method of preparation and handling of the mouse embryo fibroblasts, the mouse strain from which the fibroblasts are derived and the quality of the particular batch, may affect and may favour stem cell renewal, extraembryonic differentiation or somatic differentiation. Hence, the specification not only recognizes that the fibroblast feeder layers are important, but also recognizes that there are

subsets of the types of fibroblast feeder layer which would support controlled differentiation of the embryonic stem cells. Once a batch of fibroblast feeder cells is identified, such batch can be stored and resurrected for subsequent use in directing the induction of somatic differentiation.

The specification further provides, at the bottom of page 21, that "the modulation of stem cell growth by appropriate use of the fibroblast feeder layer and manipulation of the culture conditions provides an example whereby somatic differentiation may be induced *in vitro* concomitant with the limitation of stem cell renewal without the induction of wide spread cell death or extraembryonic differentiation." This would then favor differentiation of somatic cells as described in the following paragraph on page 22.

Applicants submit that the present inventors uniquely recognized, inter alia, that the various fibroblast batches differed in their potential to produce somatic versus extraembryonic differentiation. See page 21, line 4 to 19 of the specification. The present inventors had to screen batches to find those that favored somatic differentiation. Examples of feeder cell lines which are extremely effective in inducing somatic differentiation include B-83, which is the product of an SVJ-129 X SVJ-129 and is still in use today. On the other hand, feeder cell line B-72, which is a product of an inbred cross of SVJ-129 X SVJ-129, was marked as a throw out of fibroblasts because this particular cell line either killed the ES cells or drove the ES cells to extraembryonic differentiation.

Accordingly, Applicants respectfully submit that the specification adequately teaches screening fibroblast feeder cell lines for those that favor somatic differentiation and the use of a differentiation inducing fibroblast feeder layers (which favors somatic differentiation) to induce a differentiated somatic lineage or multiple differentiated somatic lineage under conditions that

do not permit continued stem cell renewal but do no kill stem cells or induce their unidirectional differentiation into extraembryonic lineages.

In Thompson et al., while the cells spontaneously differentiated, there was no induction or purposeful direction of the embryonic stem cells toward a somatic differentiation. Applicants respectfully submit that cells must be cultured in specific conditions as disclosed in the specification and recited in the present claims, e.g., claim 20. There is no teaching or suggestion in Thompson et al. that these specific conditions must be employed to induce somatic differentiation of the embryonic stem cells.

In summary, Applicants submit that Thompson et al. do not teach culturing cells under conditions which do not induce extraembryonic differentiation and cell death and promote proliferation of undifferentiated stem cells, which conditions are taught in the present specification and recited in the claims. Thompson et al. also do not teach culturing cells on a particular type of differentiation inducing fibroblast feeder layer which favors somatic differentiation, because Thompson did not appreciate that certain subsets of fibroblast feeder layers did in fact exist and specific fibroblast feeder layers are required to induce somatic differentiation. Therefore, Applicants respectfully submit that the present specification teaches, and the instant claims recite, conditions that are not disclosed or even recognized in Thomson et al.

Furthermore, Applicants respectfully submit that the term "inducing" by definition means prevailing upon or bringing on a result by artificial means. In the context of the present invention and in light of the specification, the term is clearly understood by those skilled in the art to mean that a condition is employed to control the outcome of the differentiation.

Specifically, the condition employed in the claimed methods is the use of differentiation inducing fibroblast feeder layers which favor somatic differentiation. In contrast, Applicants submit that Thompson et al. do not provide any enabling disclosure for methods of inducing somatic differentiation.

In the Advisory Action, the Examiner maintains that the present claims are broad and do not include any steps or parameters which are different from those taught by Thomson et al. In addition, the Examiner states that while the handling or the source of fibroblast cell line may affect its suitability as a feeder layer, there is no objective evidence of record indicating that the fibroblast layer itself uniquely impacts the differentiation of ES cells. The Examiner points out that the two fibroblast cell lines, B-83 and B-72, which were referenced in the §1.116 Response to illustrate the suitability of fibroblast cells as feeder cells, are not identified in the specification.

During the interview conducted on May 21, 2003, the Examiner acknowledged that the present invention uniquely recognizes the ability of certain fibroblast feeder cells in affecting the differentiation of stem cells, i.e., somatic differentiation vs. extraembryonic differentiation. The Examiner also acknowledged that such recognition is described generally in the specification and does not appear to be provided by any prior art. However, the Examiner requests the Applicants to provide some objective showing in support of such recognition, e.g., by way of a declaration pursuant to 37 C.F.R. §1.132. The Examiner advised us that he would consider such declaration if timely submitted. The Examiner also suggested that the claims be amended to more distinctly delineate the use of suitable fibroblast feeder cells as a principal feature of the claimed methods.

Consistent with the Examiner's suggestion, Applicants have added claims 47-57, directed to methods of inducing somatic differentiation, wherein the methods comprise

"providing a somatic differentiation-inducing fibroblast feeder layer" (emphasis added), and growing the undifferentiated, pluripotent human embryonic stem cells on the fibroblast feeder layer. Support for claims 47-52 is found in the specification, e.g., at page 20, lines 23 to 25; page 20, line 19; page 21, line 22; page 21, line 2 to 25; and in original claims, e.g., claims 21-25. Claims 53-57 depend upon claim 52 and further delineate the fibroblast feeder layer used for culturing ICM cells. Support for claims 53-57 is found throughout the specification and in original claims 10-12. No new matter is added.

In addition, Applicants are providing herewith a §1.132 Declaration of Dr. Martin F. Pera in support of the identification and use of suitable fibroblasts in favoring or directing the differentiation toward somatic lineage(s). In particular, the Declaration describes experiments in which a number of fibroblast cell lines, including B-83 and B-72, were prepared and tested for their suitability for supporting undifferentiated stem cell growth and directing differentiation towards somatic lineages. See Paragraphs 15-26 of the Declaration. As shown in Exhibit C attached to the Declaration and summarized in the table on pages 7-8 of the Declaration, some fibroblast cell lines (including B-83) did not induce spontaneous differentiation, whereas some other lines (including B-72) did induce spontaneous differentiation especially toward an extra-embryonic lineage under the otherwise identical culture conditions. B-83 was also shown to maintain the ability to support HES cell growth and direct somatic differentiation after freeze-thawing. See Paragraphs 27 -29 of the Declaration.

As Dr. Pera testified in the Declaration, the results shown in Exhibits B-E clearly illustrate that the fibroblast layer uniquely impacts the differentiation of HES cells, and that fibroblast feeder cells differ in their potential to support controlled differentiation of the embryonic stem cells into somatic lineages. Thus, identification and selection of suitable

fibroblast feeder cells permit effective induction of somatic differentiation, as opposed to differentiation into extraembryonic lineages.

As Dr. Pera further testified in the Declaration, the recognition of the varying potentials among fibroblast feeder cells in inducing somatic differentiation of ES cells and the selection of those fibroblast feeder cells that favor somatic differentiation relative to extraembryonic differentiation, are uniquely provided by the present application, and are not taught by Thomson et al. or any prior art extant.

Accordingly, Applicants respectfully submit that the presently claimed methods are not taught by Thomson et al. Withdrawal of the rejection under §102(b) based on Thomson (1998) and the rejection under §102(e) based on the '806 patent is therefore respectfully requested.

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

Frank S. DiGiglio
Registration No. 31,346

Scully, Scott, Murphy & Presser
400 Garden City Plaza
Garden City, New York 11530
(516) 742-4343
XZ:ab

Encls.: Declaration of Martin F. Pera and
Exhibits A-E